Recurrence of Herpes Simplex in the Mouse Requires an Intact Nerve Supply to the Skin

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SUMMARY

The nerves supplying the pinna of the ear of mice latently infected in the 2nd and 3rd cervical ganglia were sectioned. Immediately after neurotomy, or some days later, the denervated ears were stripped with cellophane tape to induce recurrent disease. The cervical ganglia and skin of the ears were tested for the presence of infectious virus at different times after neurotomy. Nerve section induced a low incidence of reactivation of virus in ganglia. After neurotomy, infectious virus was isolated from the skin very rarely and recurrent disease was not seen.

Carton & Kilbourne (1952) and Carton (1953) showed that section of the sensory root of the trigeminal ganglion of patients treated for trigeminal neuralgia was often followed by the appearance of herpetic lesions around the mouth. Moreover, section of nerves emanating from the ganglion prior to section of the sensory root prevented such recurrent disease. These observations strongly suggest that intact peripheral nerves are essential for the development of recurrent herpes simplex. In guinea-pigs, where herpes simplex virus (HSV) can frequently persist in the footpad (Scriba, 1977), a low incidence of spontaneous recurrent disease caused by HSV-2 has been demonstrated in denervated animals (Scriba, 1981). We therefore investigated whether intact nerve connections between latently infected ganglia and peripheral tissues are necessary for development of recurrent disease in mice, where such disease can be induced.

Four-week-old female outbred Swiss white mice were injected subcutaneously in the right ear with $6 \times 10^4$ to $3 \times 10^5$ p.f.u. HSV-1 strain SC16 (Hill et al., 1975). Since a similar incidence of latency was found with all doses within this range, results from mice given different doses are not considered separately. Five to 19 weeks after primary infection, mice with clinically normal ear skin were anaesthetized by intraperitoneal (i.p.) injection of sodium pentobarbitone, and the major sensory nerves to the right pinna (the lesser occipital and great auricular) together with the motor nerves to the muscles of the pinna (the auricular branches of the facial nerve) were sectioned at the base of the pinna. Other mice were operated on in the same way but the nerves were left intact (sham neurotomy). Immediately after the operation, the right pinna of some of the mice was stripped six times with cellophane tape, a procedure known to induce recurrent disease (Hill et al., 1978). One to 4 days after this treatment, mice were killed by i.p. injection of pentobarbitone and the 2nd and 3rd right cervical ganglia were removed and ground in 0-2 ml growth medium (199 with 5% foetal calf serum). The suspensions were then frozen and thawed thrice and put onto Vero cell cultures grown in 25 cm$^2$ tissue culture flasks. At the same time, the skin from the upper surface of the right ear was scraped off, ground in 0-5 ml growth medium, and the homogenate was put onto Vero cell cultures to isolate infectious virus. In other groups of mice, only the ganglia, or only the skin of the ear was tested for the presence of infectious virus. All cultures were observed daily for 1 week for c.p.e. characteristic of HSV.

One to 4 days after neurotomy, infectious virus was isolated from the ganglia of five of 120 mice which had been stripped immediately after the operation, and from two of 88 mice that had not (Table 1). These figures are considerably lower than those observed by Walz et al. (1974). With mice infected in the footpad, these workers found infectious virus in the lumbosacral ganglia of 20 of 66 animals after section of the sciatic nerve. However, they sectioned the nerve
Table 1. Isolation of HSV from ganglia* and skin after nerve section

<table>
<thead>
<tr>
<th>Treatment to mouse</th>
<th>Tissue sampled</th>
<th>Number of mice with virus/number tested</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>for infectious</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>virus</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Neurotomy alone†</td>
<td>Ganglia</td>
<td>1/16‡</td>
<td>0/19</td>
</tr>
<tr>
<td></td>
<td>Ear skin</td>
<td>0/30</td>
<td>0/34</td>
</tr>
<tr>
<td>Neurotomy and stripping§</td>
<td>Ganglia</td>
<td>2/16</td>
<td>1/48</td>
</tr>
<tr>
<td></td>
<td>Ear skin</td>
<td>1/32</td>
<td>1/62</td>
</tr>
<tr>
<td>Sham neurotomy and stripping</td>
<td>Ear skin</td>
<td>N.D.†</td>
<td>N.D.</td>
</tr>
<tr>
<td>Stripping alone¶</td>
<td>Ear skin</td>
<td>1/25</td>
<td>0/10</td>
</tr>
</tbody>
</table>

* From 2nd and 3rd cervical ganglia, after grinding, then freezing and thawing thrice immediately after removal from the animal.
† Lesser occipital, great auricular and auricular branches of facial nerves were cut.
‡ Number with virus/number tested.
§ Cellophane tape stripping of the skin of the right ear, within 10 min of operation.
† Not done.
¶ From Harbour et al., 1983.

much closer to the ganglion than we did, and this produces a more severe response in the neuronal soma than section at a greater distance from the cell body (Lieberman, 1974). Reactivation of HSV may be a consequence of such metabolic changes in neurons, associated with repair of damage to nerves (Price & Schmitz, 1978), but the degree of damage required for efficient reactivation is not known. The incidence of reactivation in the ganglion during the 4 days after neurotomy and stripping is not significantly different (by $\chi^2$ test) from that following stripping alone (seven of 95 mice; Harbour et al., 1983). This similarity is hardly surprising since nerve section is likely to prevent the signal produced by stripping the skin from reaching the ganglion.

When the ears were tested, virus was isolated during the 4 days after neurotomy from only one of 148 neurotomized mice, and from three of 181 animals that were also stripped (Table 1). These are significantly ($\chi^2: P < 0.001$) lower incidences than from mice whose ears were stripped immediately after sham neurotomy and tested for virus on the third day after the operation. In other experiments (Harbour et al., 1983), of 56 mice stripped without nerve section, eight yielded virus 1 to 4 days after stripping, a result significantly higher ($\chi^2: P < 0.001$) than in mice stripped after neurotomy. The amount of virus detected in the skin of the ears of the four neurotomized mice (1, 5, 20, 100 p.f.u./ear) was lower than in sham-neurotomized or intact animals (2 p.f.u./ear, 9 p.f.u./ear, 4 mice with about 50 p.f.u./ear, 7 with > 100 p.f.u./ear). However, since the number of mice with virus was small, interpretation of the yields of virus is difficult.

When mice were stripped immediately after neurotomy, the inflammation in the skin of the ear was so severe that it was not possible to judge whether the mice developed recrudescent herpes simplex. However, if the right ears of the mice were stripped 4 days after neurotomy, tissue damage was limited and by microscopic examination (Hill et al., 1982) it was possible to see specific herpetic lesions. Of 28 such mice, none developed specific lesions whereas without operation 19 of 77 (25%) mice developed lesions after the skin of their ears was stripped (Hill et al., 1982). In addition, of a total of 52 mice stripped 4 days after neurotomy, none yielded virus from the skin of the ears or 5 days after stripping. By contrast, virus was isolated from the skin of six of 22 mice stripped 4 days after sham neurotomy and sampled 5 days after stripping. The lack of virus isolation after neurotomy was not due to elimination of latent virus from the ganglia since 24 of 28 (86%) mice in which the nerves were sectioned 7 weeks after infection were found to harbour virus in their ganglia 1 week after neurotomy. The method of demonstrating latent infection has been described elsewhere (Harbour et al., 1981).

The origin of the virus isolated from the ears of mice after neurotomy is not known. However, there are minor nerve supplies to the pinna from the trigeminal ganglion and from the jugular ganglion of the vagus nerve. In other mice infected originally in the ear, we found latent
infection with HSV in the trigeminal ganglion of five of 41 (12%) of mice, and in the jugular ganglion in the same proportion of a different group, again of 41 animals. Thus, it is possible that the virus found in the ears of the mice after section of the cervical nerves originated in these ganglia. Alternatively, since we have shown by organ culture (Hill et al., 1980) that up to 10% of latently infected animals without recurrent disease have virus in their ears at any given time, the virus might already have been in the skin before nerve section. The trauma produced by surgery and stripping may have been sufficient to enhance the growth of such virus so that it was detected in homogenized tissue without the need for organ culture.

The experiments reported in this paper are the first to show that an intact nerve supply between a latently infected ganglion and the corresponding peripheral site is one essential for the development of recurrent herpes simplex. Hence, support is given to the hypothesis that it is the virus which is reactivated from latency in the ganglion which is largely responsible for recurrence of infectious virus in the peripheral tissue and thereby, in some circumstances, the production of recurrent disease.

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REFERENCES


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